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(54) Title: HUMANIZED ANTIBODY AGAINST CD18 (57) Abstract A humanized antibody having all or part of the CDRs as defined and capable of binding to the human CD-18 antigen. The antibody is of use in therapy in treating leukocyte mediated conditions such as inhibiting ingress of leukocytes into the lung and other organs and treatment of inflammation.		

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HUMANIZED ANTIBODY AGAINST CD18

The present invention relates to an antibody which binds to the CD18 antigen, to the preparation of such an antibody and to a pharmaceutical composition which contains the antibody.

5 Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. 10 Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant 15 domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

 The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the 20 light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases 25 forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. CDRs and framework regions of 30 antibodies may be determined by reference to Kabat et al

logical interest" US Dept.

in EP-A-0239400. The CDRs may be derived from a rat or mouse monoclonal antibody. The fram work of the variable domains, and the constant domains, of the altered antibody may be derived from a human antibody. Such a humanised antibody elicits a negligible immune response when administered to a human compared to the immune response mounted by a human against a rat or mouse antibody. Humanised CAMPATH-1 antibody is disclosed in EP-A-0328404 (Campath is a Trade Mark of The Wellcome group of companies).

According to one aspect of the present invention, there is provided a humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human CD18 antigen:

light chain: CDR1 (SEQ ID NOS: 3 and 4)
 CDR2 (SEQ ID NOS: 5 and 6)
 CDR3 (SEQ ID NOS: 7 and 8)
heavy chain: CDR1 (SEQ ID NOS: 11 and 12)
 CDR2 (SEQ ID NOS: 13 and 14)
 CDR3 (SEQ ID NOS: 15 and 16)

According to another aspect the invention provides a DNA molecule encoding a humanised antibody in which sufficient of the amino acid sequence of each CDR shown above is provided such that the antibody is capable of binding to the human CD-18 antigen.

The antibody preferably has the structure of a natural antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a (Fab')₂ fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG such as IgG1, IgG2, IgG3 or IgG4; or IgM, IgA, IgE or IgD. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain.

The antibody may be a chimeric antibody of the type

described in WO 86/01533. A chimeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain and/or heavy chain variable domain. Typically the chimeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused to the C-terminus of the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from a protein having known binding specificity, from a protein toxin or indeed from any protein expressed by a gene. The non-immunoglobulin region may be a carbohydrate region. The two regions of the chimeric antibody may be connected via a cleavable linker sequence.

The light chain CDRs 1 to 3 and heavy chain CDRs 1 to 3 of SEQ ID NOS: 3 to 8 and SEQ ID NOS: 11 to 16 respectively are the CDRs of the YFC51.1.1 rat antibody which is a CD18 antibody. The specificity of a humanised antibody for the human CD18 antigen can be determined by flow cytometry, monocyte adhesion and/or by T-cell proliferation assays as follows:

Monocyte (MNC) Adhesion

MNC's are treated with the phorbol diester PDBu (10^{-9} M) in the presence and absence of antibody ($20\mu\text{l}$) for 5 minutes. These cells are then transferred to bovine aortic endothelial cell (BAEC) monolayers and incubated for 30 minutes in a humidified atmosphere of 95% air, 5% CO_2 at 37°C . Non-adherent cells are removed by washing in phosphate buffered saline (PBS) three times. The adherent cells are then lysed in situ with $50\mu\text{l}$, 0.5% hexadecyltrimethyl

an increase in absorbance. The optical density of the samples can then be recorded at 450nm using a multi-well plate reader (Anthos series, Lab Teck instruments). Comparisons can then be made between treated and untreated samples (Bath et al., J. Immunol. Meth., 118, 59-65, (1989)).

Flow cytometry

Surface labelling of rat, rabbit, guinea-pig and human monocytes with antibody is carried out according to the method of Gladwin et al., (Biochim. Biophys. Acta., 1052, 166-172 (1990)). Briefly, 1 ml aliquots of cells suspension (5×10^6) are incubated with the appropriate antibody, monodispersed and incubated on melting ice for 30 minutes. The cells are twice washed in PBS and incubated for a further 30 minutes with a 1:200 dilution of rabbit anti-rat F(ab'), FITC conjugate on melting ice. The cells are finally washed three times in PBS and fixed in 0.1% para-formaldehyde. Analysis of surface labelling can be performed using an Epics Elite flow cytometer (Coulter cytometry, Hialeah, FL) using standard computer, electronics and optics. The Elite is configured with a 15mW 488nm Argon-ion laser (Cyomics model 2201, San Jose, CA). Monocyte and lymphocyte populations are separated by forward angle light scatter and side scatter. Green fluorescence data for 2×10^4 monocytes is collected using bit-map gating and collected on a three decade log scale. Green fluorescence data for 2×10^4 neutrophils is collected in a similar manner. For each sample, mean fluorescence intensity in the presence of the primary mAb is compared with cells incubated with rabbit anti-rat F(ab'), FITC fragments alone and the percentage labelling of the cells determined. Samples can be labelled in triplicate and

T-cell proliferation assay

Human mononuclear cells are prepared from defibrinated blood using density gradient separation over Ficoll-paque. Lymphocytes (2×10^5 cells) are cultured in each well of a flat bottomed 96-well microtitre plate (Nunc, Roskild, Denmark), in RPMI 1640 supplemented with 10% autologous serum, 2mM glutamine and 100iU penicillin/100 μ g ml⁻¹ streptomycin. Triplicate cultures are set up with the medium alone or with antigen (Tetanus Toxoid, 3 μ g ml⁻¹) or mitogen (PHA, 1 μ g ml⁻¹), in the presence or absence of different concentrations of monoclonal antibodies. Cells are cultured at 37°C in a humidified atmosphere of 95% air, 5% CO₂ for five days. Wells are then pulsed with 1 μ Ci [methyl³H] thymidine (2Ci mmol⁻¹, Amersham), harvested 18 hours later and radioactivity counted by liquid scintillation using a B counter (LKB, Betaplate, Sweden). The results are expressed as mean \pm SEM.

Suitably, the CDRs of a humanised antibody are the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 above. The amino acid sequences of these CDRs may be changed, however. The amino acid sequence of each CDR may be changed by up to 40% by amino acid substitutions, insertions and/or deletions, for example by up to 30%, up to 20% or up to 10%.

Each CDR may therefore include one or two amino acid substitutions, insertions and/or deletions. There may be up to three amino acid substitutions, insertions, and/or deletions in light chain CDR. Up to four amino acid substitutions, insertions and/or deletions may be present in light chain CDR1 or heavy chain CDR3. Up to six amino

Preferably the framework of the variable region of the antibody heavy chain is substantially homologous to the corresponding framework of the human protein NEWM (Saul *et al.*, J. Biol. Chem. 25, 585-597, (1987)). Homology in
5 respect of the framework is generally 80% or more with respect to NEWM, for example 90% or more or 95% or more. A number of amino acid substitutions, insertions and/or deletions may be present. Candidate framework changes that may be made to restore binding include changes of amino
10 acid residues 27, 30, 48, 66, 67, 71, 91, 93 and 94. The amino acid numbering is according to Kabat *et al.*

The framework of the variable region of the antibody light chain is typically substantially homologous to the variable domain framework of the protein REI (Epp *et al.*,
15 Eur. J. Biochem. 45, 513-524, (1974)). Homology in respect of the framework is generally 80% or more with respect to REI, for example 90% or more or 95% or more. A number of amino acid substitutions, insertions and/or deletions may be present, for example at amino acid residue 71 according
20 to the numbering of Kabat *et al.*

A humanised antibody is prepared according to the invention by a process which comprises maintaining a host transformed with a first expression vector which encodes the light chain of the humanised antibody and with a second
25 expression vector which encodes the heavy chain of the humanised antibody under such conditions that each chain is expressed and isolating the humanised antibody formed by assembly of the thus-expressed chains.

The first and second expression vectors may be the same vector. The invention further provides:
30

ding the light chain or the heavy

replacement. The CDRs of a variable region of a light or heavy chain of a human antibody are replaced by sufficient of the amino acid sequence of each CDR of the rat anti-human-CD18 antibody YFC51.1.1 such that the resulting antibody is capable of binding to the CD18 antigen. The CDR-encoding regions of DNA encoding a hypervariable region of a human antibody chain are replaced by DNA encoding the desired CDRs. If appropriate, this altered DNA is linked to DNA encoding a constant domain for the antibody chain. The DNA is cloned into an expression vector. The expression vector is introduced into a compatible host cell which is cultured under such conditions that the antibody chain is expressed. Complementary antibody chains which are co-expressed in this way may then assemble to form the humanised antibody.

The present invention is described herein with particular reference to the production of a humanised antibody having CDRs derived directly or indirectly from the rat antibody YFC51.1.1. However the techniques described herein can equally be used to derive other humanised anti CD-18 antibodies. According to a further aspect, the present invention provides a humanised (CDR grafted) anti CD-18 antibody.

There are four general steps to humanise a monoclonal antibody. These are:

- (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains;
- (2) designing the humanised antibody, i.e. deciding which antibody framework region to use during the

Step 1: Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To humanise an antibody only the amino acid sequence of antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody's variable domain amino acid sequence is from cloned cDNA encoding the heavy and light chain variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains. In the present instance, the nucleotide sequence and predicted amino acid sequence of the light and heavy chains of the rodent YFC51.1.1 antibody are shown in SEQ ID NOS: 1 and 2 and SEQ ID NOS: 9 and 10.

Step 2: Designing the humanised antibody

There are several factors to consider in deciding which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework

variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s). A suitable human antibody variable domain sequence can be selected as follows:

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if only human immunoglobulin sequences are included.

2. List the human antibody variable domain sequences and compare for homology. Primarily the comparison is performed on length of CDRs, except CDR3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent antibody CDR to one of the human subgroups as a first

Step 3: The actual humanising methodologies/techniques

An antibody may be humanised by grafting the desired CDRs onto a human framework according to EP-A-0239400. A DNA sequence encoding the desired reshaped antibody can therefore be made beginning with the human DNA whose CDRs it is wished to reshape. The rodent variable domain amino acid sequence containing the desired CDRs is compared to that of the chosen human antibody variable domain sequence. The residues in the human variable domain are marked that need to be changed to the corresponding residue in the rodent to make the human variable region incorporate the rodent CDRs. There may also be residues that need substituting in, adding to or deleting from the human sequence.

Oligonucleotides are synthesized that can be used to mutagenize the human variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesizer one has available. The method of oligonucleotide-directed in vitro mutagenesis is well known.

Alternatively, humanisation may be achieved using the recombinant polymerase chain reaction (PCR) methodology of WO 92/07075. Using this methodology, a CDR may be spliced between the framework regions of a human antibody.

In general, the technique of WO 92/07075 can be performed using a template comprising two human framework regions, AB and CD, and between them, the CDR which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers

Thus, the amplified regions AB and CD may undergo gene splicing by overlap extension to produce the humanised product in a single reaction.

5 Step 4: The transfection and expression of the reshaped antibody

Following the mutagenesis reactions to reshape the antibody, the mutagenised DNAs can be linked to an appropriate DNA encoding a light or heavy chain constant region, cloned into an expression vector, and transfected
10 into host cells, preferably mammalian cells. These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising:

(a) preparing a first replicable expression vector
15 including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a human antibody and the CDRs required for the humanised antibody of the invention;

20 (b) preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

25 (c) transforming a cell line with the first or both prepared vectors; and

(d) culturing said transformed cell line to produce said altered antibody.

Preferably the DNA sequence in step (a) encodes both the variable domain and the or each constant domain of the
30 human antibody chain. The humanised antibody can be prepared using any suitable recombinant expression system. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or

immortalised mammalian cell line, which is

also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

The CHO cells used for expression of the antibodies according to the invention may be dihydrofolate reductase (dhfr) deficient and so dependent on thymidine and hypoxanthine for growth (Urlaub *et al.*, Proc. Natl. Acad. Sci. U.S.A., 77 4216-4220 (1980)). The parental dhfr CHO cell line is transfected with the DNA encoding the antibody and dhfr which enables selection of CHO cell transformants of dhfr positive phenotype. Selection is carried out by culturing the colonies on media devoid of thymidine and hypoxanthine, the absence of which prevents untransformed cells from growing and transformed cells from resalvaging the folate pathway and thus bypassing the selection system. These transformants usually express low levels of the DNA of interest by virtue of co-integration of transfected DNA of interest and DNA encoding dhfr. The expression levels of the DNA encoding the antibody may be increased by amplification using methotrexate (MTX). This drug is a direct inhibitor of the enzyme dhfr and allows isolation of resistant colonies which amplify their dhfr gene copy number sufficiently to survive under these conditions. Since the DNA sequences encoding dhfr and the antibody are closely linked in the original transformants, there is usually concomitant amplification, and therefore increased expression of the desired antibody.

Another preferred expression system for use with CHO or myeloma cells is the glutamine synthetase (GS) amplification system described in WO 87/04462. This system involves the transfection of a cell with DNA encoding the enzyme GS and with DNA encoding the desired antibody. Cells are then selected which grow in glutamine free medium and can thus be assumed to have integrated the DNA encoding

5 GS. These selected clones are then subjected to inhibition of the enzyme GS using methionine sulfoximine (Msx). The cells, in order to survive, will amplify the DNA encoding GS with concomitant amplification of the DNA encoding the antibody.

10 Although the cell line used to produce the humanised antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. In particular, it is envisaged that E. coli - derived bacterial strains could be used. The antibody obtained is checked for functionality. If functionality is lost, it is necessary to return to step (2) and alter the framework of the antibody.

15 Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be recovered and purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, 20 column chromatography, gel electrophoresis and the like (See, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most 25 preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, a humanised antibody may then be used therapeutically or in developing and performing assay procedures, immunofluorescent stainings, and the like (See, generally, Immunological 30 Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The humanised CD18 antibodies can be used for example in the treatment of leukocyte mediated conditions. The humanised CD18 antibodies typically find use in inhibiting 35 influx of leukocytes into the lungs and other organs during sepsis or other infectious or non-infectious trauma. The

humanised CD18 antibody can therefore be used for inhibiting the ingress of leukocytes into the lung and other organs in patients having endotoxic shock or adult respiratory distress syndrome. The antibody can be used to treat asthma or leukocyte-mediated reperfusion damage post thrombolytic therapy, to treat inflammation in the lung and other organs in patients having an inflammation caused by sepsis or other infectious or non-infectious trauma, to eliminate or reduce inflammation in a patient being administered with an anti-infective agent or to assist in the administration of a therapeutic drug to a patient during chemotherapy (EP-A-0346078).

The humanised antibodies of the present invention may also be used in combination with other antibodies, particularly human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the so-called "Clusters of Differentiation" as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard, et al., Eds., Springer-Verlag, N.Y. (1984).

The antibodies can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

An antibody of the present invention may form part of an immunotoxin. Immunotoxins are characterized by two components and are particularly useful for killing selected

to a particular cell type, such as cells comprising a carcinoma. Th two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet", Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982).

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). See, generally, "Chimeric Toxins," Olsnes and Phil, *Pharmac. Ther.*, 25, 335-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985).

The delivery component of the immunotoxin is a humanised antibody according to the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgA, IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The invention further provides a pharmaceutical composition comprising a pharmaceutically acceptable

carrier or diluent and, as active ingredient, a humanised antibody according to the invention. The composition may comprise an immunotoxin according to the invention. The humanised antibody, immunotoxin and pharmaceutical compositions thereof of this invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously.

The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to

ed., Mack Publishing Company, Easton, Pennsylvania (1980).

5 The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, 10 IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

15 The compositions containing the present human-like antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications. An amount adequate to 20 accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the infection and the general state of the patient's own immune system, but generally range from about 1 to about 200 mg of antibody per dose, with dosages of 25 from 5 to 25 mg per patient being more commonly used. It must be kept in mind that the materials of the invention may generally be employed in serious disease states, that is life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of 30 extraneous substances and the lower probability of "foreign

administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose". In this use, the precise amounts again depend upon the patient's
5 state of health and general level of immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per patient. A preferred prophylactic use is for the prevention of kidney transplant rejection.

Single or multiple administrations of the
10 compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

15 Human-like antibodies of the present invention can further find a wide variety of utilities in vitro. By way of example, the exemplary antibodies can be utilized for T-cell typing, for isolating specific CD18 antigen-bearing cells or fragments of the receptor, for vaccine
20 preparation, or the like.

For diagnostic purposes, the antibodies may either be labelled or unlabelled. Unlabelled antibodies can be used in combination with other labelled antibodies (second
25 antibodies) that are reactive with the humanised antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labelled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme
30 substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject
35 antibodies in the protection against or detection of a cellular activity or for the presence of a selected antigen. Thus, a humanised antibody of the present

inv ntion may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above. The kit will generally also include a set of instructions for use.

The following Example illustrates the invention.

EXAMPLE

Cloning and sequencing of the YFC51.1.1 rat anti-human-CD18 heavy and light chains

Total RNA was isolated from 2.5×10^7 YFC51.1.1 expressing cells following the method of Chomczynski and Sacchi (Anal. Biochem., 162, 156-159, (1987)), using 1ml of extraction solution per 1×10^7 cells. The resulting RNA pellet was redissolved in 50 μ l diethyl pyrocarbonate (DEPC)-treated distilled water, and spectrophotometrically determined to be at a concentration of 4.4 μ g/ μ l.

cDNA was synthesized from the isolated mRNA and cloned into the plasmid pSPORT-1 using the SUPERScript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (BRL) following the method recommended by the manufacturer.

5 Escherichia coli MAX EFFICIENCY DH5 α Competent Cells (BRL) were transformed with the resulting cDNA/pSPORT-1 ligation. Approximately 5000 colonies were lifted onto Hybond-N nylon filters (Amersham) and lysed, denatured and fixed following the method of Buluwela et al (Nucleic Acids Res., 17, 452,

10 (1989)). The filters were treated with proteinase K (50 μ g/ml) in 0.2 x SSC, 0.1% SDS at 55°C for 30 min, and then excess debris removed with a tissue.

(i) Heavy chain

An oligonucleotide as shown in SEQ ID NO: 17 complementary to a portion of rat gamma-CH1 constant region (bases 496-515) was end-labelled and used to screen the filters for YFC51.1.1 heavy chain following the standard protocols. Approximately 50 potential positive colonies were detected, and 20 selected for further analysis.

15 Plasmid DNA was prepared using the method of Del Sal et al (Nucleic Acids Res., 16, 9878, (1988)) and 12 of the 20 contained inserts of the expected size for rat immunoglobulin heavy chain cDNA. A clone, p51H.6, was selected, and the variable region sequenced in both

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following the dideoxy chain

following the manufacturer's protocol. Approximately 40 potential positive colonies were detected, and 24 selected for further analysis. Plasmid DNA was prepared as described above. Both Y3-Ag 1.2.3 and YFC51.1.1 light chains were isolated (Y3 cell line being hybridoma fusion partner) but were distinguishable by having different restriction patterns. One clone, p51L.4, containing the YFC51.1.1 light chain was chosen and sequenced as described for the heavy chain. The sequence of the variable region is shown in SEQ ID NOS: 1 and 2.

Designing the humanised antibody

Using the selection procedure described in Step (2) above, the human variable domain frameworks of the NEWM heavy chain and REI light chain (Kabat et al., 1987) were chosen for the humanisation process.

Construction of the humanised heavy and light chain genes

The humanised heavy and light chains were constructed following the method of Lewis and Crowe (Gene, 101, 297-302, (1991)).

(i) Light Chain

Light chain oligonucleotide primers:

- A_L: SEQ ID NO: 18:
- 25 B_L: SEQ ID NO: 19:
- C_L: SEQ ID NO: 20:
- D_L: SEQ ID NO: 21:
- E_L: SEQ ID NO: 22:
- F_L: SEQ ID NO: 23:
- 30 G_L: SEQ ID NO: 24:
- H_L: SEQ ID NO: 25:

PCR reactions (Saiki et al., Science 239, 487-491, (1988)) were performed in a programmable heating block

The initial template for the PCR was CAMPATH-1H heavy chain. The rodent CDR's were grafted on to the template following the recombinant PCR method as described above, but using oligonucleotide primers A_H to H_H. The final PCR, i.e. fragments AD_H and EH_H with primers A_H and H_H, did not give a high yield of product so a fragment AF_H was generated (from fragments AD_H and EF_H) and used with fragment EH_H in a PCR with primers A_H and H_H. Oligonucleotides A_H and H_H were designed with HindIII and EcoRI sites respectively to enable initial cloning of the humanised variable region, and a SpeI site was introduced into the NEWM framework 4 (FR4) region of oligonucleotide G_H to facilitate subsequent cloning of the variable region with a suitable constant region of choice. The SpeI site was chosen so as not to alter the leucine residue at position 109 (numbering according to Kabat et al, 1987) of the humanised heavy chain template. Four out of the six human heavy J-minigenes possess a leucine at this position (Kabat et al, 1987). Thus the use of the engineered SpeI site should be generally applicable.

The humanised heavy chain variable region recombinant PCR product was cloned into HindIII/EcoRI-cut pUC-18 (BRL), and plasmid isolates of the correct sequence were chosen. The FR4 and $\gamma 1$ constant regions of CAMPATH-1H heavy chain were PCR cloned into pUC-18 (BRL) using oligonucleotide primers X_H (SEQ ID NO: 34) and Y_H (SEQ ID NO: 35). Primer X_H contains SpeI and HindIII sites, and Y_H an EcoRI site. The HindIII and EcoRI sites were used to clone the PCR product into pUC-18, and plasmid isolates of the correct sequence were selected. The complete heavy chain was subsequently reconstituted from the humanised variable region and $\gamma 1$ constant region clones using the

DNA encoding the humanised heavy and light chains were cloned into the vectors pEE6.hCMV and pEE12 respectively, see Stephens & Cockett, Nucleic Acids Res., 17, 7110 (1989); Bebbington et al., Biotechnology, 10, 169 (1992); and Bebbington and Hentschel in Glover ed., DNA Cloning Volume III, Academic Press (1987). The vector pEE12 is a pBR322 - based vector containing the h-CMV-MEI promoter and the hamster glutamine synthetase (GS) cDNA under control of the SV40 early region promoter. The vector pEE12 corresponds to pEE6 (see EP-A-0338841) with the GS cDNA expression cassette driven by the SV40 promoter transcribing in the same direction as the h-CMV-MEI promoter. Cells transfected with the vectors pEE6, hCMV and pEE12 are capable of growth in glutamine free medium because of the presence of the GS cDNA. As the selection is only on the pEE12 plasmid, effective expression relies upon co-integration of both plasmids.

The recombinant plasmids (5 μ g of each) were transfected into 5x10⁵ COS-1 cells using the Transfectam reagent (Promega, Southampton, U.K.) under the conditions recommended by the manufacturer. Stock COS-1 cells (source ECACC, Porton Down, U.K.) were maintained in DMEM medium (Flow, Irvine, U.K.) supplemental with 10% foetal calf serum (APP, Dudley, U.K.). COS cell transfections were carried out in DMEM medium (Flow, Irvine, U.K.). Growth media from COS-1 cells four days post transfection were assayed by a sandwich ELISA assay using flexible microtitre plates (Falcon, Becton-Dickinson, Plymouth, U.K.) coated with polyclonal anti-human IgG (Sigma, Poole, U.K.) as capture antibody. The assay sample was added and detection

for FACS analysis according to the method of Gladwin et al,
Biochem. Biophys. Acta, 1052, 166-172 (1990). Briefly
100 μ l aliquots of a cell suspension (10^6) were incubated
with the appropriate antibody (spent COS cell supernatant)
5 and incubated on melting ice for 30 minutes. The cells
were washed twice in PBS and incubated for a further 30
minutes with the appropriate second antibody (see below).
The cells were washed again and 1:50 dilutions of anti-rat
Ig-FITC or anti-human Ig-FITC conjugates were added on
10 melting ice. Finally, the cells were washed three times in
PBS and fixed in 0.1% paraformaldehyde. Analysis of
surface labelling was performed using a Becton-Dickenson
FACScan using standard computer, electronics and optics.

The humanised antibody in the COS cell supernatant
15 was shown to bind MF-14 cells as well as inhibiting the
binding of the rat YFC51.1.1 monoclonal antibody. Since
the humanised antibody was shown to have retained binding
for CD18 by blocking the binding of the rat monoclonal
antibody, stable NS0 transfectants were generated.

20

Stable expression in NS0 cells

A single expression vector for generating stable
transfectants of NS0 cells was generated by cloning the
complete heavy chain expression cassette from pEE6 into the

25

BamHI site of the pEE6 vector.

nitrate but with sodium pyruvate at 110 mg/l (GIBCO/BRL, Paisley, U.K.) supplemented with 1X non-essential amino acids (Flow, Irvine, U.K.) 2mM glutamine (GIBCO) and 10% foetal calf serum (APP, Dudley, U.K.). NSO cells were centrifuged, washed and re-suspended in cold PBS, such that after the addition of the DNA the cells would be at a concentration of 10^7 cells/ml. The linearised plasmid DNA, 40 μ g, was added to 10^7 cells in an electroporation cuvette on ice. The cells and DNA were mixed gently so as to avoid generating bubbles and the mixture was left on ice for 5 minutes. The outside of the cuvette was wiped dry and two consecutive pulses at 1500V, 3mF were delivered using a Gene Pulser (Bio-Rad). The cuvette was returned to ice for 5 minutes.

Transfected cells were transferred to 96 well plates at densities of 3×10^5 , 7.5×10^4 and 1.5×10^4 cells/ml in 50 μ l of non-selective medium and incubated at 37°C for 24 hours. Subsequently 100 μ l of selective DMEM medium (i.e. without glutamine and ferric nitrate but with sodium pyruvate at 100 mg/l (GIBCO/BRL, Paisley, U.K.) supplemented with glutamate (60 mg/ml), asparagine (60 mg/ml; Sigma, Poole, U.K.), 1X non-essential amino acids, 7 mg/l of adenosine, cytidine, guanosine and uridine, 2.4 μ g/ml of thymidine (Sigma Poole, U.K.) and 10% dialysed

cells/well in 100 μ l of medium and incubated overnight. 100 μ l of selective medium containing a concentration of L-methionine sulfoximine (MSX) was added. MSX is a toxic glutamine analogue that allows for selection of vector amplification. Each 96-well plate had a different final concentration of MSX, ranging from 200 μ M down to 12.5 μ M. Individual colonies were isolated from each independent transfectant at the highest MSX concentration at which MSX resistance occurred. The colonies were expanded and antibody secretion rate (in μ g/10⁶ cells/day) was compared with the unamplified rate. Clones were obtained that expressed the humanised antibody at 1 to 3 μ g/10⁶ cells/day.

The humanised antibody was purified from spent tissue culture supernatant by affinity chromatography over a Superose protein-G column (Pharmacia) and used in T-cell proliferation assays and Clq binding studies.

T-cell Proliferation

Peripheral human mono-nuclear cells were isolated from defibrinated whole human blood using Lymphoprep (Nycomed, Oslo, Norway) and following the manufacturer's protocol. Triplicate cultures were set up in 96 well flat bottomed microtitre plates (Nunc, Roskild, Denmark) with the medium clone (RPMI 1640 supplemented with 10% autologous serum, 2mM glutamine and 100 IU/ml penicillin, 100 μ g/ml streptomycin) or with medium and antigen (Tetanus

T-cell response but had little effect on the PHA induced proliferation. However, at high levels of antibody (50 μ g/ml) and low levels of PHA (2.5 μ g/ml) up to 80% inhibition could be obtained.

5

Complement binding

Human mononuclear cells (prepared as above) were stimulated with PHA at 5 μ g/ml and incubated at 37°C for 3 days. The PHA was removed by washing the cells in PBS.

10 The cells were then incubated with 10 μ g/ml of test antibody for 20 minutes on ice, cells washed in ice cold PBS and incubated with ice cold human serum for 20 minutes. The human serum was removed by washing in ice cold PBS. The cells were then incubated for 20 minutes with a

15 fluoreceinated polyclonal sheep anti-human C1q. Unbound anti-C1q was removed by washing cells in PBS and cells were analysed on a Becton-Dickenson FACScan. YFC51.1.1 was found to bind human C1q weakly and no binding was detected for the humanised antibody. Potential therapeutic uses for

20 anti-CD18 antibodies rely on transient inhibition of CD18-mediated adherence of leukocytes rather than depletion of CD18 positive cells. Accordingly the inability of the humanised antibody to fix human complement on CD18 positive cells is an advantage since it suggests that in vivo the

25 antibody will not deplete using complement but will

antibody and a FITC-labelled anti-human or anti-rat antibody added. Unbound label was removed by washing and the cells were analysed on a Becton-Dickenson FACScan. Pre-incubation of MF14 cells with 10 μ g/ml of YFC51.1.1 antibody completely blocked the binding of 0.1 μ g/ml of humanised antibody. In the reciprocal experiment, pre-

5

SEQUENCE LISTING

(1) INFORMATION FOR SEQ ID NO : 1

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 375 base pairs
(B) TYPE : nucleic acid
10 (C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

15 (vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

20

(A) NAME/KEY : CDS
(B) LOCATION : 1..375
(D) OTHER INFORMATION : /product= "Variable region
light chain"
25 /standard_name= "YFC51.1.1"

(ix) FEATURE:

(D) OTHER INFORMATION : /function= "CDR 1"

(ix) FEATURE:

5 (A) NAME/KEY : misc_feature
(B) LOCATION : 208..228
(D) OTHER INFORMATION : /function= "CDR 2"

(ix) FEATURE:

10 (A) NAME/KEY : misc_feature
(B) LOCATION : 325..351
(D) OTHER INFORMATION : /function= "CDR 3"

15 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 1:

ATG AGG GTC CAG GTT CAG TTT CTG GGG CTC CTT CTG CTC TGG ACA TCA 48
Met Arg Val Gln Val Gln Phe Leu Gly Leu Leu Leu Leu Trp Thr Ser
1 5 10 15

20 GGT GCC CAG TGT GAT GTC CAG ATG ACC CAG TCT CCG TCT TAT CTT GCT 96
Gly Ala Gln Cys Asp Val Gln Met Thr Gln Ser Pro Ser Tyr Leu Ala
20 25 30

25 GCG TCT CCT GGA GAA AGT GTT TCC ATC AGT TGC AAG GCA AGT AAG AGC 144
Ala Ser Pro Gly Glu Ser Val Ser Ile Ser Cys Lys Ala S r Lys Ser
35 40 45

ATT AGC AAT TAT TTA GCC TCC TAT CAA CAG AAA CCT CCC GAA GCA AAT 182

AAC CTG GAG CCT GCA GAT TTT GCA GTC TAC TAC TGT CAA CAG TAT TAT 336
 Asn Leu Glu Pro Ala Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
 100 105 110

5

GAA AGA CCG CTC ACG TTC GGT TCT GGG ACC AAG CTG GAG 375
 Glu Arg Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu
 115 120 125

10 (2) INFORMATION FOR SEQ ID NO : 2

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH : 125 amino acids
 (B) TYPE : amino acid
 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

20 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 2:

Met Arg Val Gln Val Gln Phe Leu Gly Leu Leu Leu Leu Trp Thr Ser
 1 5 10 15
 Gly Ala Gln Cys Asp Val Gln Met Thr Gln Ser Pro Ser Tyr Leu Ala
 20 25 30
 Ala Ser Pro Gly Glu Ser Val Ser Ile Ser Cys Lys Ala Ser Lys Ser
 35 40 45
 Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Glu Ala Asn
 50 55 60
 Lys Leu Leu Val Tyr Tyr Gly Ser Thr Leu Arg Ser Gly Ile Pro Ser
 65 70 75 80
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg
 85 90 95
 Asn Leu Glu Pro Ala Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
 100 105 110

Glu Arg Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu
115 120 125

(3) INFORMATION FOR SEQ ID NO : 3

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 33 base pairs
(B) TYPE : nucleic acid
10 (C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

15 (vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

20

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..33
(D) OTHER INFORMATION : /function= "CDR 1"

25

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..33

30

(xi) SEQUENCE DATA

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 11 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

10 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 4:

Lys Ala Ser Lys Ser Ile Ser Asn Tyr Leu Ala
1 5 10

15 (5) INFORMATION FOR SEQ ID NO : 5

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH : 21 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus
30 (ix) FEATURE:

(A) NAME/KEY : misc_feature

35

(A) NAME/KEY : CDS
(B) LOCATION : 1..21

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 5:

5

TAT GGG TCA ACT TTG CGA TCT
Tyr Gly Ser Thr Leu Arg Ser
1 5

21

10

(6) INFORMATION FOR SEQ ID NO : 6

(1) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH : 7 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

20

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 6:

Tyr Gly Ser Thr Leu Arg Ser
1 5

25

(7) INFORMATION FOR SEQ ID NO : 7

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH : 27 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

35

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

36

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

5 (A) NAME/KEY : misc_feature
(B) LOCATION : 1..27
(D) OTHER INFORMATION : /function= "CDR 3"

(ix) FEATURE

10 (A) NAME/KEY : CDS
(B) LOCATION : 1..27

15 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 7:

CAA CAG TAT TAT GAA AGA CCG CTC ACG

27

Gln Gln Tyr Tyr Glu Arg Pro Leu Thr

1 5

20

(8) INFORMATION FOR SEQ ID NO : 8

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH : 9 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

30 (ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 8:

Gln Gln Tyr Tyr Glu Arg Pro Leu Thr

35 1 5

(9) INFORMATION FOR SEQ ID NO : 9

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 417 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

15 (A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..417
20 (D) OTHER INFORMATION : /product= "Heavy chain
variable region with
signal sequence"
/standard_name "YFC51.1.1"

25 (ix) FEATURE:

(A) NAME/KEY : misc_signal
(B) LOCATION : 1..57
30 (D) OTHER INFORMATION : /function= "Signal
sequence"

(ix) FEATURE:

35 (A) NAME/KEY : misc_feature
(B) LOCATION : 148..162
(D) OTHER INFORMATION : /function= "CDR 1"

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 205..255

5

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 352..384

10

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 9:

15	ATG AAA TGC AGC TGG ATC AAC CTC TTC TTG ATG GCA CTA GCT TCA GGG	48
	Met Lys Cys Ser Trp Ile Asn Leu Phe Leu Met Ala Leu Ala Ser Gly	
	1 5 10 15	
20	GTC TAC GCA GAA GTG CAG CTG CAA CAG TCT GGG CCC GAG CTT CGG AGA	96
	Val Tyr Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Arg Arg	
	20 25 30	
25	CCT GGG TCC TCA GTC AAG TTG TCT TGT AAG ACT TCT GGC TAC AGC ATT	144
	Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Ser Ile	
	35 40 45	
30	AAA GAT TAC CTT CTG CAC TGG GTA AAA CAT AGG CCA GAA TAC GGC CTG	192
	Lys Asp Tyr Leu Leu His Trp Val Lys His Arg Pro Glu Tyr Gly Leu	
	50 55 60	
35	GAA TGG ATA GGA TGG ATT GAT CCT GAG GAT GGT GAA ACA AAG TAT GGT	240
	Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly	
	65 70 75 80	
40	CAG AAG TTT CAA AGC AGG GCC ACA CTC ACT GCA GAT ACA TCC TCC AAC	288
	Gln Lys Phe Gln Ser Arg Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn	
	85 90 95	
	ACA GCC TAC ATG CAA CTC AGC AGC CTG ACG TCT GAC GAC ACA GCA ACC	336
	Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Asp Asp Thr Ala Thr	
	100 105 110	
	TAT TTT TGT ACT AGA GGC GAA TAT AGA TAC AAC TCG TGG TTT GAT TAC	384

Tyr Phe Cys Thr Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
 115 120 125

5 TGG GGC CAA GGC ACT CTG GTC ACT GTC TCT TCA 417
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 130 135

(10) INFORMATION FOR SEQ ID NO : 10

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 139 amino acids
 (B) TYPE : amino acid
 (D) TOPOLOGY : linear

15

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 10:

20 Met Lys Cys Ser Trp Ile Asn Leu Phe Leu Met Ala Leu Ala Ser Gly
 1 5 10 15

Val Tyr Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Arg Arg
 20 25 30

25

Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Ser Ile
 35 40 45

30 Lys Asp Tyr Leu Leu His Trp Val Lys His Arg Pro Glu Tyr Gly Leu
 50 55 60

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly
 65 70 75 80

35 Gln Lys Phe Gln Ser Arg Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn
 85 90 95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Asp Asp Thr Ala Thr
 100 105 110

40

Tyr Phe Cys Thr Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr

40

115

120

125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

130

135

5

(11) INFORMATION FOR SEQ ID NO : 11

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH : 15 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

15

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

20

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

25

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..15
(D) OTHER INFORMATION : /function= "CDR 1"

(ix) FEATURE:

30

(A) NAME/KEY : CDS
(B) LOCATION : 1..15

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 11:

35

GAT TAC CTT CTG CAC
Asp Tyr Leu Leu His

15

1

5

(12) INFORMATION FOR SEQ ID NO : 12

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 5 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

10

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 12:

15 Asp Tyr Leu Leu His
1 5

(13) INFORMATION FOR SEQ ID NO : 13

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 51 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

25

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

30

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

35 (A) NAME/KEY : misc_feature
(B) LOCATION : 1..51
(D) OTHER INFORMATION : /function= "CDR 2"

(ix) FEATURE:

5 (A) NAME/KEY : CDS
(B) LOCATION : 1..51

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 13:

10 TGG ATT GAT CCT GAG GAT GGT GAA ACA AAG TAT GGT CAG AAG TTT CAA 48
Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly Gln Lys Phe Gln
1 5 10 15
AGC 51
Ser

15

(14) INFORMATION FOR SEQ ID NO : 14

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH : 17 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 14:

30 Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly Gln Lys Phe Gln
1 5 10 15
Ser

35 (15) INFORMATION FOR SEQ ID NO : 15

(i) SEQUENCE CHARACTERISTICS:

43

(A) LENGTH : 33 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

5

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

10 (A) ORGANISM : Rattus rattus

(ix) FEATURE:

15 (A) NAME/KEY : misc_feature
(B) LOCATION : 1..33
(D) OTHER INFORMATION : /function= "CDR 3"

(ix) FEATURE:

20 (A) NAME/KEY : CDS
(B) LOCATION : 1..33

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 15:

25 GGC GAA TAT AGA TAC AAC TCG TGG TTT GAT TAC
Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
1 5 10

33

30 (16) INFORMATION FOR SEQ ID NO : 16

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH : 11 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 16:

5 Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
1 5 10

(17) INFORMATION FOR SEQ ID NO : 17

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20 bases
(B) TYPE : nucleic acid
15 (C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

20 (iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(vi) ORIGINAL SOURCE

25

(A) ORGANISM : Rattus Rattus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 17:

30 AGTGGATAGA CAGATGGGGC

20

(18) INFORMATION FOR SEQ ID NO : 18

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30 bases

45

(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

5 (ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

10

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 18:

GATCAAGCTT CTCTACAGTT ACTGAGCACA

30

15

(19) INFORMATION FOR SEQ ID NO : 19

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH : 43 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

30

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 19:

46

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 43 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

10 (iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 20:

15

AGAGCATTAG CAATTATTTA GCCTGGTACC AGCAGAAGCC AGG

43

(21) INFORMATION FOR SEQ ID NO : 21

20

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH : 41 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(22) INFORMATION FOR SEQ ID NO : 22

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 41 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

15 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 22:

TATGGGTCAA CTTGCGATC TGGTGTGCCA AGCAGATTCA G

41

20

(23) INFORMATION FOR SEQ ID NO : 23

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH : 47 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

30 (ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

35 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 23:

48

CGTGAGCGGT CTTTCATAAT ACTGTTGGCA GTAGTAGGTG GCGATGT

47

(24) INFORMATION FOR SEQ ID NO : 24

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH	: 47 bases
(B) TYPE	: nucleic acid
(C) STRANDEDNESS	: single
(D) TOPOLOGY	: linear

10

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

15

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 24:

20

CAACAGTATT ATGAAAGACC GCTCACGTTT GGCCAAGGGA CCAAGGT

47

(25) INFORMATION FOR SEQ ID NO : 25

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH	: 30 bases
(B) TYPE	: nucleic acid
(C) STRANDEDNESS	: single
(D) TOPOLOGY	: linear

30

49

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 25:

5 GATCAAGCTT CTAACACTCT CCCCTGTTGA

30

(26) INFORMATION FOR SEQ ID NO : 26

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 31 bases

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

15 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

20

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 26:

25 TGGGATCGAT CAAGCTTTAC AGTTACTGAG C

31

(27) INFORMATION FOR SEQ ID NO : 27

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36 bases

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

35 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

50

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

5 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 27:

GTGCAGAAGG TAATCGGTGA AGGTGAAGCC AGACAC

36

10 (28) INFORMATION FOR SEQ ID NO : 28

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH : 36 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

20 (ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

25 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 28:

GATTACCTTC TGCACTGGGT GAGACAGCCA CCTGGA

36

30 (29) INFORMATION FOR SEQ ID NO : 29

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH : 54 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

51

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

5 (iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 29:

10 ATACTTTGTT TCACCATCCT CAGGATCAAT CCATCCAATC CACTCAAGAC CTCG 54

(30) INFORMATION FOR SEQ ID NO : 30

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 54 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

20

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

25 (iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 30:

30 GGTGAAACAA AGTATGGTCA GAAGTTTCAA AGCAGAGTGA CAATGCTGGT AGAC 54

(31) INFORMATION FOR SEQ ID NO : 31

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 45 bases
(B) TYPE : nucleic acid

52

(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

5

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

10 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 31:

CCACGAGTTG TATCTATATT CGCCTCTTGC ACAATAATAG ACCGC

45

15 (32) INFORMATION FOR SEQ ID NO : 32

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 54 bases

20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

25

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

30 (xi) SEQUENCE DESCRIPTION : SEQ ID NO: 32:

AGATACAACT CGTGTTTGA TTACTGGGGT CAAGGCTCAC TAGTCACAGT CTCC

54

35 (33) INFORMATION FOR SEQ ID NO : 33

(i) SEQUENCE CHARACTERISTICS:

53

(A) LENGTH : 36 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

5

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

10 (iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 33:

TAGAGTCCTG AGGGAATTCG GACAGCCGGG AAGGTG

36

15

(34) INFORMATION FOR SEQ ID NO : 34

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH : 48 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

25

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

30 (iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 34:

GCTGCTCCTT TTAAGCTTTG GGGTCAAGGC TCACTAGTCA CAGTCTCC

48

35

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 33 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

10 (iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 35:

15 AAGCTTCCGT CGAATTCATT TACCCGGAGA CAG

CLAIMS:

1. A humanised antibody in which sufficient of the
5 amino acid sequence of each CDR shown below is provided
such that the antibody is capable of binding to the human
CD-18 antigen:

light chain: CDR1 (SEQ ID NOS: 3 and 4)

CDR2 (SEQ ID NOS: 5 and 6)

10 CDR3 (SEQ ID NOS: 7 and 8)

heavy chain: CDR1 (SEQ ID NOS: 11 and 12)

CDR2 (SEQ ID NOS: 13 and 14)

CDR3 (SEQ ID NOS: 15 and 16).

2. An antibody as claimed in claim 1, in which the
15 variable domain framework of the light chain is or is
substantially homologous to the variable domain framework
of the protein REI.

3. An antibody as claimed in claim 1 or 2, in which
the variable domain framework of the heavy chain is or is
20 substantially homologous to the variable domain framework
of the protein NEWM.

4. An antibody as claimed in any one of claims 1 to
3 in which the CDRs are the light chain CDRs 1 to 3 and the
heavy chain CDRs 1 to 3 specified in claim 1.

25 5. A process for the preparation of a humanised
antibody as defined in any of claims 1 to 4, which process
comprises providing a host transformed with either (i) a
first expression vector which encodes the light chain of
the humanised antibody and a second expression vector which
30 encodes the heavy chain of the humanised antibody; or (ii)
a single expression vector which encodes both the light

which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human CD-18 antigen:

light chain: CDR1 (SEQ ID NOS: 3 and 4)

5 CDR2 (SEQ ID NOS: 5 and 6)

CDR3 (SEQ ID NOS: 7 and 8)

heavy chain: CDR1 (SEQ ID NOS: 11 and 12)

CDR2 (SEQ ID NOS: 13 and 14)

CDR3 (SEQ ID NOS: 15 and 16).

10 7. A DNA molecule as claimed in claim 6, in which the variable domain framework of the light chain is or is substantially homologous to the variable domain framework of the protein REI.

15 8. A DNA molecule as claimed in claim 6 or 7, in which the variable domain framework of the heavy chain is or is substantially homogenous to the variable domain framework of the protein NEWM.

20 9. A DNA molecule as claimed in any one of claims 6 to 8 in which the CDRs are the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 specified in claim 6.

10. A DNA molecule as claimed in any of claims 6 to 9 in the form of an expression vector.

11. A host transformed with an expression vector as claimed in claim 10.

25 12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, a humanised antibody as defined in any of claims 1 to 4.

INTERNATIONAL SEARCH REPORT

International Application

PCT/GB 92/01289

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 C12N15/13; C12P21/08; C07K15/28; C12N5/10 A61K39/395		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12N ; C12P ; C07K ; A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claims No. ¹³
X	NUCLEIC ACIDS RESEARCH vol. 19, no. 9, 11 May 1991, LONDON, GB pages 2471 - 2476 B. DAUGHERTY ET AL. 'Polymerase chain reaction facilitates the cloning, CDR-grafting, and rapid expression of a murine monoclonal antibody directed against the CD18 component of leukocyte integrins.' see the whole document <div style="text-align: center; margin-top: 20px;"> --- -/-- </div>	1-12
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search <div style="text-align: center; margin-top: 10px;"> 30 SEPTEMBER 1992 </div>	Date of Mailing of this International Search Report <div style="text-align: center; margin-top: 10px;"> 19. 10. 92 </div>	
International Searching Authority <div style="text-align: center; margin-top: 10px;"> EUROPEAN PATENT OFFICE </div>	Signature of Authorized Officer <div style="text-align: center; margin-top: 10px;"> NOOIJ F.J.M. </div>	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	<p>GENE vol. 101, no. 2, 30 May 1991, AMSTERDAM, THE NETHERLANDS pages 297 - 302 A. LEWIS ET AL. 'Immunoglobulin complementarity-determining region grafting by recombinant polymerase chain reaction to generate humanised monoclonal antibodies.' cited in the application see the whole document</p> <p>---</p>	1-12
Y	<p>EP,A,0 346 078 (THE ROCKEFELLER UNIVERSITY) 13 December 1989 cited in the application see claims</p> <p>---</p>	1-12
Y	<p>(EDS. W. KNAPP ET AL.) 'Leukocyte Typing IV. White cell differentiation antigens' 1989, OXFORD UNIVERSITY PRESS, OXFORD see page 1079</p> <p>---</p>	1-12
Y	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, no. 10, 15 May 1991, WASHINGTON DC, US pages 4181 - 4185 S. GORMAN ET AL. 'Reshaping a therapeutic CD4 antibody.' see abstract</p> <p>---</p>	1-12
P,X	<p>EP,A,0 438 312 (MERCK & CO., INC.) 24 July 1991 see claims</p> <p>-----</p>	1-12

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 9201289
SA 62146

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 30/09/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0346078	13-12-89	AU-B- 620100	13-02-92
		AU-A- 3608489	14-12-89
		JP-A- 2104534	17-04-90
EP-A-0438312	24-07-91	AU-A- 6984391	25-07-91
		CA-A- 2034574	20-07-91
		EP-A- 0440351	07-08-91